Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Simonovich VA, Burgos Pratx LD, Scibona P, et al. A randomized trial of convalescent plasma in Covid-19 severe pneumonia. N Engl J Med. DOI: 10.1056/NEJMoa2031304

No effect of Convalescent Plasma in Covid-19 severe pneumonia: The PlasmAr Trial

This supplement contains the following items:

[1] Version 1.0 was an internal protocol draft. Version 2.0 is the first one approved by the Institutional Review Board (IRB) of Hospital Italiano de Buenos Aires on May 4th 2020, which supported the beginning of the study.

[2] Version 3.0 was approved by the IRB on July 16th 2020, before de interim analysis

[3] This statistical analysis plan corresponds to the first approved protocol version 2.0

[4] This statistical analysis plan corresponds to the approved protocol version 3.0

Part I. A:
Study Protocol (version 2.0)

Convalescent plasma in patients with Covid-19 severe pneumonia; a multicenter, randomized, placebo controlled clinical trial.

1. Introduction

Since spanish flu in 1918, convalescent plasma has been used for the treatment of some infections with the assumption that passive immunization can provide the amount of neutralizing antibodies necessary to control the evolution of the disease while establishing the patient's immune response [1]. This strategy was also used more recently in treating the severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), influenza A (H1N1) in 2009, avian influenza (H5N1) and Ebola, with different results.

There is previous experience in the use of convalescent plasma in patients with Argentine Hemorrhagic Fever (ALF) in our country. In a double-blind study carried out by the Institute of Virology of Pergamino between 1974 and 1977, which included 217, and 188 were finally randomized, there was a significant decrease in mortality [2]. Among the conclusions it is worth highlighting that the administration within 8 days of the onset of symptoms as a determining factor.

Table 1 shows the primary outcome from the Argentine Hemorrhagic Fever study [2]

TABLE I—MORTALITY IN PATIENTS WITH AHF TREATED WITH IMMUNE OR NORMAL PLASMA

Treatment	Total cases	Improved	Died	Mortality (%)
Immune plasma	91	90	1	1·1
Normal plasma	97	81	16	16·5
Total	188	171	17	—

χ_1^2=13·53; p<0·01

At present, given the rapid expansion of the epidemic of SARS by coronavirus 2 (SARS-CoV-2) known as Covid-19, different scientific groups and government authorities are working on different measures and regulations that favor the implementation of experimental treatments based on pathophysiological mechanisms or supported by low quality evidence [3].

In March 2020, a first report from *Shen et al*. based on the experience in five patients with COVID-19 treated successfully with convalescent plasma, raised worldwide expectations regarding its clinical role in fighting this new disease [4,5]. However, an editorial in the same journal raised important arguments about the real efficacy of this therapeutic strategy, mentioning that, although the cases reported by *Shen et al*. were adequately presented and well studied, the research had important limitations, highlighting that it was not possible to

determine the real clinical effect of the plasma intervention and / or ensure that the patients had recovered as a result of this intervention, since other therapeutic options such as corticosteroids and antivirals had also been applied [5]. Another controversial point would be the ideal time for the administration of convalescent plasma. In this case, the plasma infusion was carried out 3 weeks after hospital admission, and its effect on earlier administration was not clear [45].

Another study that provides evidence on the use of convalescent plasma and the timing of its administration is the non-randomized study presented by Cheng et al in 2005. Reinforcing what was reported by Maiztegui et al, the study showed that the time of plasma administration was statistically different between the groups, giving a relevant role to this variable. Age and specific antibody development were other statistically important variables influencing prognosis of these patients. Patients who had a positive reverse-transcriptase-polymerase-chain-reaction (RT-PCR) and negative coronavirus serology at the time of convalescent plasma administration, had better outcomes than those who were already seropositive (66.7% vs 20% p = 0.001). In the multivariate analysis, only the convalescent plasma administration time and the positive RT-PCR were statistically significant [6].

2. Study Proposal

2.1 Study proposal and hypothesis

General purpose of the study: to test the efficacy and safety of convalescent plasma for the treatment of Covid19 pneumonia

Study Hypothesis: Convalescent Plasma significantly improves outcome of severe Covid-19 pneumonia in patients

2.2 Study design

The PlasmAr study is a multicenter, double-blind, placebo-controlled. The randomization will be asymmetric 2:1.

2.2.1 Rational basis of the intervention

There is no specific therapy of proven effectiveness against Covid-19. Several therapeutics lines are currently being studied internationally. In this context, the administration of antibodies through the transfusion of convalescent plasma appears as a reasonable option for research. This strategy has already been recommended as empirical treatment during the Ebola and MERS epidemics[4], and within the context of clinical research in severe influenza illness. Recently, a series of uncontrolled cases of patients with Covid-19 who improved their clinical condition after the administration of convalescent plasma was published. Currently, several clinical studies with convalescent plasma are being evaluated or already implemented in different regions of the world [5 6].

2.2.2 Rationale for the use of Placebo control arm

The specific effectiveness of the use of convalescent plasma in the treatment of Covid-19 pneumonia is not known. Although the case series and reports offer encouraging results, there is no high-quality evidence on this regard. In addition, the methodology for plasma collection, administration and control is relatively complex and its efficacy should be convincingly established before promoting it for healthcare use.

Controlled clinical trials of the use of hyperimmune immunoglobulin in patients with severe influenza did not confirm the results that previous observational studies had suggested. The overall risk of pool plasma transfusion of patients is very low. Beyond this, it has been clearly recommended to prioritize the application of therapeutic strategies in the context of clinical studies over the empirical use of treatments with as yet unproven efficacy [7 8].

In the case of the present study, the intervention strategy is proposed in an “add on” modality, as eventual antiviral treatment already been initiated, may not preclude the addition of convalescent plasma, since it is a completely different type of therapeutic approach. For this reason, the participation of patients in the present study will not condition their opportunity to receive other types of treatments, both in the plasma and in the placebo arms.

2.3 Study population

Patients with a confirmed diagnosis of Covid-19 pneumonia hospitalized in any of the participating institutions, who present the inclusion criteria of the study and voluntarily provide their informed consent.

2.4 Scope of the study and participating institutions

The multicenter clinical trial will be carried out at the Hospital Italiano de Buenos Aires and other centers in Ciudad Autónoma de Buenos Aires, Buenos Aires province and other provinces.

INMUNOVA S.A. will provide the logistics for the standardization of the antibody content of the convalescent plasma and the determination of the neutralizing antibody titer.

2.5 Study aims

2.5.1 Primary outcomes

We will analyse the difference in patient's clinical status day 30 after intervention, represented by one of the following six mutually exclusive ordinal categories [9]:
1- death
2- invasive ventilatory support
3- hospitalized with supplemental oxygen requirements
4- hospitalized without supplemental oxygen requirements
5- discharged without full return of baseline physical function
6- discharged with full return of baseline physical function

2.5.2 Secondary outcomes

1. 6 categories ordinal outcome on day 7th after intervention.
2. 6 categories ordinal outcome on day 14th after intervention.
3. Time from intervention to discharge from hospital (in days).
4. Time from intervention to discharge from the ICU (in days).

5. Time from intervention to death (in days)
6. Time from the intervention to complete restitution of physical functions (according to baseline status).
7. Percentage of participants with adverse events / serious adverse events.
8. Percentage of participants with negative PCR at day 14.
9. Serum D-dimer (ng/ml) plasma concentration at day 14.
10. Serum Ferritin (ng/ml) plasma concentration at day 14.
11. Plasma concentration of total antibodies on day 2 after the intervention.
12. Plasma concentration of total antibodies on day 7 after the intervention.
13. Percentage of post-transfusion specific adverse reactions between groups.

2.6 Inclusion/exclusion criteria of the patients

To enter the study, patients must meet the inclusion criteria and none of the exclusion criteria. Patients who can potentially be admitted to the study will receive the usual treatments and may be receiving other specific treatments for COVID-19

2.6.1 Inclusion criterias

- Patients older than 18 years
- Confirmed diagnosis of Covid-19 by qualitative reverse transcriptase-polymerase chain reaction (qRT-PCR -GeneDX Co, Ltd or similar).
- Diagnosis of pneumonia by imaging (Rx or CT).
- One or more of the following criteria:
    - Score MSOFA (Modified SOFA) [10] with a score of 2 or higher
    - Oxygen saturation less than 93% breathing at room air
    - PaFi (Ratio of arterial oxygen pressure over inspired fraction of oxygen) less than 300
- Provision of informed consent by the participant

Table 2: MSofa score [10]

Modified Sepsis-related Organ Failure Assessments (MSOFA)

Score	0	1	2	3	4
Respiration PaO_2(mmHg) /FiO_2 SPO_2/FiO_2 (%)	>400 >400	<400 <400	<300 ≤ 315	<200 ≤ 235 With Respiratory support	<100 ≤ 150 With Respiratory support
Liver	No scleral icterus or jaundice			Scleral icterus or jaundice	
Cardiovascular Hypotension *ug/kg/min for 1 hour	MAP≥70	MAP <70	*Dopamine <5 or *Dobutamine (any dose)	*Dopamine5.1-15 or *Adrenaline≤0.1 *Norepinephrine≤0.1	*Dopamine>15 or *Adrenaline>0.1 *Norepinephrine>0.1
CNS Glasgow Coma Scale	15	13-14	10-12	6-9	<6
Renal Creatinine (mg/dL) Creatinine (umol/L) Urine Output (mL/day)	<1.2 <106	1.2-1.9 106-170	2.0-3.4 177-301	3.5-4.9 309-433 <500	>4.9 >433 <200
MSOFA mortality: 0-7=4%; 8-11=31% ; ≥11=58%			**SOFA mortality: 0-7=5%; 8-11=28% ; ≥11=53%**		

Adapted from Grissom, CK, Brown, SM, Kuttler, KG, et alA modified sequential organ failure assessment score for critical care triage. *Disaster Med Public Health Prep.* 2010;4 (4):277–284

Prepared by Malaysia Sepsis Alliance (MySepsis) & Universiti Kebangsaan Malaysia Medical Center. Link: www.mysepsis.org

2.6.2 Exclusion criterias

- Pregnancy
- People of reproductive age who do not agree to avoid having unprotected sex until day 30 after the start of the study
- Breastfeeding women
- Patients who have received experimental treatments under development within 30 days prior to admission
- Patients with a history of allergic reactions to blood transfusions or its components
- Confirmation of another concomitant microbiological cause of pneumonia other than Covid-19
- Patients who are on mechanical ventilation, with multiple organ failure or who for any other reason cannot voluntarily give their consent.

2.7 Clinical procedures

The patients will be selected by physicians of intensive care units, intermediate care units and infectious diseases department.

The Informed Consent process will be carried out in accordance with the provisions of point 5 of this protocol.

The investigator will again review the inclusion / exclusion criteria of the patients based on data from the physical examination and laboratory parameters.

Then the patient will be randomized.

The following table details the schedule of procedures to be performed on each patient included in the study.

Table 3: clinical procedures schedule

Procedures	**day 0**	**day 1**	**day 2**	**day 3**	**day 4-6**	**day 7**	**day 8-13**	**day 14**	**day 15-29**	**day 30**
Inclusion/exclusion criteria	X									
SARS-CoV-2 RT-PCR	X							X		
Informed consent	X									
Physical exam	X	X	X	X	X	X	X	X	X	X
Body mass index	X									
Heart/respiratory rate	X	X	X	X	X	X	X	X	X	X
SpO2/Fio2	X	X	X	X	X	X	X	X	X	X
Temperature (C°)	X	X	X	X	X	X	X	X	X	X
MSofa score	X					X		X		
Intervention	X									
Primary outcome		X	X	X	X	X	X	X	X	X
Concomitant drugs	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X
Neutralizing antibody testing	X		X			X		X		
Total antibody testing	X		X			X		X		
Ferritin level	X							X		
D-dimer level	X							X		
Complete blood count exams	X			X		X		X		
Chemistry exams	X			X		X		X		
Pregnancy test	X									X

The procedures detailed in the protocol will be performed within the context of the hospitalization of participants. In addition to the procedures indicated by the protocol each of the participating centers will perform the procedures and analytical exams that they consider necessary for the best care of patients.The procedures may be carried out by telephone, with the exception of the procedures of days 7, 14 and 30. These procedures must be carried out through a face-to-face visit, either at institution or at the patient's home or place of stay, including blood draws corresponding to days 7 and 14. During all the development of the study but in particular in case of early hospital discharge, the need for all participants of childbearing age to maintain measures of effective contraception will be enforced.

2.7.1 Convalescent plasma safety issues

The safety assessment of the administration of plasma is described in point 6. Regarding the safety of plasma in terms of apheresis and safety measures, the recommendations of the Ministerio de Salud de la Nación will be followed and those in force by the accreditations of the Hospital Italiano de Buenos Aires as described in point 2.7.2

2.7.2 Convalescent plasma/placebo infusion: schedule and administration route

Once included in the study, the patient will be monitored and the infusion will begin. The plasma / placebo will be infused intravenously, preferably through one of the branches of a central vein catheter, if the patient had one in place to ensure complete infusion of the plasma / placebo. If not, it is recommended that it be the safest route available to the patient.

The calculation of the volume to be transfused will be 5-10ml / kg adjusting the volume to body weight of each patient. An administration rate of 5-10 ml/kg/h is recommended, although the final rate could be adapted in accordance with the patient's tolerance and/or risk of volume overload. No standardized premedication is required before transfusion. Patients must be clinically monitored throughout the entire transfusion process in order to assist and register any incidental adverse reaction.

2.7.3 Patient follow up

During the plasma / placebo infusion, vital signs and symptoms of adverse reactions, including acute hypersensitivity reactions, should be monitored. In the event of an emergency, we will proceed according to the emergency management protocols validated in each institution. The patient will be cared for, giving him life support and adequate treatment.

2.8 Analysis plan and statistical methodology

2.8.1 Randomization

The randomization process will consist of two stages: randomization and allocation concealment.

For the random assignment, a random list will be generated in blocks of variable size, stratified for each of the participating centers. Recruitment will be consecutive until the proposed sample size is reached. A list of random numbers will be drawn up for each of the participating centers, consigning the assignment to the branch "Intervention" (Convalescent Plasma) or "Placebo" (saline solution).

This list will remain hidden from both the doctors and nurses in charge of the assistance of each participant. The result of the assignment will be reported from the central protocol coordination structure to the head of Transfusion Medicine of each of the institutions. Those responsible for Transfusion Medicine or Hemotherapy of each center will prepare the container containing the plasma or placebo, as appropriate. Since the plasma solution is slightly opaque, the container and IV line will be covered with a colored sleeve to maintain the masking.

The statistical team and the person in charge of transfusional medicine will be unblinded to intervention. The rest of the researchers will remain blinded to the intervention branches of the study.

2.8.2 Statistical analysis

2.8.2.1 Sample size calculation

It was assumed that 52% of patients in the control arm would be in categories 6 or 5 at 30 days compared to 66% in the CP group. To achieve 80% power to detect a proportional odds ratio between arms of at least 1.8 in the clinical ordinal scale at the 0.05 (2-sided) level of significance, a sample size of 333 patients (222 in plasma arm and 111 in placebo arm) was defined. Odds ratio greater than 1.0 correspond to more favourable outcomes on CP use compared with placebo.

2.8.2.2 Statistical methodology

We included all randomized patients in the analysis according to the randomization arm (intention-to-treat analysis). We anticipate that all patients will receive the once time

intervention, but if this does not occur, the possibility of performing a per-protocol analysis is contemplated. In all cases we used the total number of participants who contributed values. Categorical variables will be presented as absolute and relative frequency in percentage. Continuous variables will be summarized as mean and standard deviation (sd) or median and interquartile interval (*IQR*) according to the observed distribution. We will apply logarithmic transformations for continuous variables with asymmetric distribution. We will apply the Wilcoxon rank sum test to compare the distribution of continuous and logarithmic transformed variables between exposure arms.

We will evaluate the association between convalescent plasma or placebo and the ordinal primary outcome, using an ordinal logistic regression model . We will use this model to estimate a common proportional odds ratio (OR) for the ordinal categories of the primary outcome between arms on the 7th, 14th and 30th day [11,12]. The proportional odds ratio assumption is evaluated using the Brant parallel regression assumption test [13,14].

We will use Cox proportional hazard regression model to evaluate time to death and time to clinical improvement and to estimate the Hazard Ratios (HR). We will use the Kaplan Meier method to estimate the cumulative incidence as a function of time. We will use Fine and Gray regression models considering death as a competing event to estimate the subHazard Ratios (sHR) for the association between arm of exposure and time to discharge from hospital, discharge from the ICU, complete restitution of physical functions, and start of invasive ventilatory support. We will use logistic regression models to estimate the OR for the comparison of adverse events between arms. All association measures will be presented with 95% confidence intervals (95%CI).

Planned subgroup analysis will be performed according to: age groups, gender, time/delay from onset of symptoms to intervention, presence of comorbidities (chronic obstructive pulmonary disease, obesity, immunosuppression, diabetes, hypertension, cardiovascular disease), baseline participant antibody titer and recruiting sites and corticosteroid concomitant treatment. We will present the interaction test *p* values with the estimated OR for each stratum. For comparisons of secondary outcomes, we will consider statistically significant p values of less than 0.05.

3. Study Intervention

3.1 Technical procedures: plasma preparation

3.1.1 Identification and recruitment of potential donors

- The identification of plasma donors may be as follows:

  - Patients hospitalized for COVID-19 will be invited to donate plasma after recovery and when they meet the inclusion criteria.

  - A list of potential donors will be obtained from the registry of convalescent patients.

  - Contact convalescent patients by phone

- All donors must have a negative PCR for SARS- CoV-19 prior donor
- Informed consent process
- Complete the "blood and / or blood component donor eligibility" questionnaire
- The donor will be asked to collect samples for pre-donor studies, serology, molecular biology (HIV-HBV-HCV) and immunohematology of apheresis pre-donor samples to titrate specific antibodies by ELISA .
- In order to guarantee traceability, they will be incorporated into a system that ensures traceability in each participating center.
- On the day of extraction, a volume that does not exceed 15% of total blood volume will be obtained from each donor.
- Units of convalescent plasma will be identified with a label that clearly identifies these units.
- The plasma will be stored at 4 degrees until the infusion, if the infusion is carried out within 48 hours of its collection
- The plasma will be frozen at -80 degrees and stored at -40 degrees until the infusion, if the infusion is carried out after 48 hours from its collection
- The place of storage will be in a specific compartment identified as “Convalescent Plasma SARS-CoV -2 "

3.1.2 Inclusion criteria for plasma donors

- General acceptance criteria for blood donors according to Administrative and Technical Regulations RM 797/13 - 139/14 - 1507/15. Directorate of Blood and Hemoderivatives of the Ministry of Health of the Nation, Argentine Association of Hemotherapy, Immunohematology and Cell Therapy (AAHITC).
- Age: 18 to 60 years.
- People who have recovered from the SARS-CoV-2 infection
- Previously diagnosed for COVID-19 and subsequently negative or SARS-CoV-2 and for other respiratory viruses

- The donor must complete a period of 28 days for complete resolution of symptoms and a negative result for covid (quali PCR swab or viral load in blood) according to FDA recommendations. If the result of this PCR is positive, perform a new assessment according to the criteria of the treating physician
- Multiparous donors must be negative for anti-HLA antibodies. If the determination of anti-HLA antibodies cannot be carried out, multiparous donors will not be accepted.
- The specific titer of total antibodies should be> 1/1000
- Study profile of transfusion transmissible infections (TTI) must be negative for hepatitis B virus, hepatitis C virus, HIV, syphilis, brucellosis, HTLV and Chagas.
- The donor must read, understand and voluntarily sign the informed consent for Apheresis Plasma Donation.

An immunohematological profile of the donated blood will be carried out according to standard practice (ABO/Detection of Irregular Antibodies/quantitative determination of Isohemagglutinins). The blood group of all recipients will be requested to analyze compatibility with the plasma pool.

Between 400 and 600 mL of plasma will be obtained from each donor using Continuous Flow and/or discontinuous cell separators validated and approved by ANMAT (Ex: Trima Accel, Terumo, Spectra optia Terumo, COMTEC), or through validated manual procedures that comply with the required standards of traceability and biosafety adjusting to current regulations.

These inclusion criteria are taken to minimize the risk of administering COVID19-infected plasma to patients, despite the fact that there is literature that ensures that the use of plasma with possible infection would not be adding burden to patients with a diagnosis.

3.1.3 Convalescent plasma

- Caption on Convalescent Plasma container: Caution. New drug: limited to investigational use.
- Plasma pools of up to 5 to 10 donors will be made in force in order to homogenize the intervention.
- Plasma pools can be used regardless of compatibility group, if the anti A and / or Anti B antibodies present a titer no greater than 1/64 according to the guidelines of the Argentine Association of Immunohematology Hemotherapy and Cellular therapy.
- If a pool has Anti A titer> 1/64, it can only be transfused in patients of group O and B according to the clinical practice guidelines of the Argentine Association of Hemotherapy Immunohematology and Cell Therapy.
- If a pool presents Anti B titer> 1/64, it can only be transfused in patients with group O

and A according to the clinical practice guidelines of the Argentine Association of Hemotherapy Immunohematology and Cell Therapy.

The plasma will be labeled as COVID-19 Convalescent Plasma appearing as directed in the traceability system for designated recipient

3.2 Methodology for the analysis of total and neutralizing antibodies

The commercial laboratory kit that has the approval of regulatory agencies for the dosage of total antibodies will be used for this project. These commercial kits will also be validated in the central laboratory of the Hospital Italiano de Buenos Aires. Once these kits have been validated, they can be used by all the centers participating in the present study.

Regarding neutralizing antibodies, Inmunova will provide a neutralizant antibody detection kit and will be validated at Italian Hospital central laboratory.

3.3 Neutralizing antibody dosing calculation

A pool will be made with plasma donations and the total antibody titer will be measured to ensure minimum antibody titers.

Measurement of neutralizing antibodies will be performed as soon as the test is available and validated. For this purpose, an aliquot of plasma from each patient will only be frozen for future measurement.

There is no information on what is the minimum neutralizing antibody titer for this disease.

It will ensure that the patient receives a plasma transfusion that has at least a total antibody titer of 1: 1000.

The calculation of the volume to be transfused will be 10 to 15 ml / kg (ensuring the minimum volume recommended by the literature and adjusting the volume to the body weight of each patient).

3.3.1 Infusion product: convalescent plasma/placebo

- The plasma will be labeled Convalescent Plasma / COVID-19 Placebo as directed in the designated recipient traceability system.
- To maintain the masking of the study, both the bag and the tubing will be covered.
- Plasma will be infused immediately to patients who meet the inclusion criteria for this treatment.

- If necessary, the plasma can be frozen as Fresh Frozen Plasma.

3.4 Procedures for unmasking assignment

The intervention will remain blind to the research subjects and to the research team throughout the entire protocol. The transfusional medicine team and the persons in charge of the statistical analysis will not be blind regarding the assignment of the branch to which the patients have been randomized.

Opening of the blinding will only be contemplated in the event that a patient dramatically worsens clinical condition, and the treating physician considers that the patient could benefit from a plasma transfusion. If unmasking is asked, a Clinical Evaluation Committee may be consulted, which will define the relevance of opening the blind. This Committee will be made up of three physicians designated for this purpose. The case will be analyzed and the committee's recommendation will not be binding but will give the treating physician support to better consider the case. The doctor must ask the transfusional medicine team to open the blind to know the branch to which the patient was assigned.

The assessment of the patient will take into account the treatments established, the possibility and access to other treatments, comorbidities and the general condition of the patient.

The analysis that will be given to the data of patients whose blind has been opened is detailed in the statistical analysis section.

4. Case report form and schedule of activities

The epidemiological, demographic, clinical, laboratory, treatment and results data will be extracted from the clinical history of each patient. The data will be collected using a standardized data collection form which will be implemented electronically through the RedCap® platform.

5. Informed consent form

All patients included in the study must give their consent as well as plasma donors. Both models of Informed Consent Forms are attached. 5.1

5.1 Procedures for informed consent in participants

5.1.1 Procedures for informed consent in included patients

All patients enrolled in the study will be hospitalized with an indication for isolation due to their pathology. The consent process can be adapted to the conditions of each of the centers. The consent process will be carried out according to the following steps:

- Once the patient is identified, the doctor delegated to take consent will sign it, putting the time at which it is done.
- The delegate physician will deliver to the assistance personnel who are in possession of a Personal Protective Equipment (PPE) for their task and they will deliver it to the patient. After having read the informed consent, if the patient has any questions, they can make a phone call to the doctor who signed the consent (these patients usually have their personal phone in their possession). The patient signs the consent after the explanations (he/she will put the time at which he signs).
- Once the consent is signed, it is considered contaminated and will be placed in a plastic bag. This process will take place in the patient's room. The assistance personnel who put the consent on the bag, will put it in a paper envelope that will be held by the person who is outside the room. This envelope will be considered clean.
- In this paper envelope, the initials of the patient and the date on which it was consented will be labeled. Later it will be filed and unarchived 7 days after the signature.
- The entire process will be described in the patient's medical record in a contemporary way.
- In the event that the patient is considered vulnerable by the researcher or the doctor delegated to take consent, a witness will be added to the video call, who will be asked to sign the consent later. Details regarding Who the person was, what links them to the patient, their identification number and the phone from which they call will be recorded in the medical record when the process is described.

5.2 Procedures for informed consent in plasma donors

5.2.1 Patients who have NOT participated in the PlasmAr study prior to discharge

Patients who meet the selection criteria for a convalescent plasma donor will be invited to participate in the study, according to previously established procedures. They will be given the information sheet and the informed consent so that they can read it with confidence. Then they will be contacted by phone by doctors from the research team delegated for the taking of informed consent to talk about the procedure and evacuate the donor's doubts.

If the potential donors are willing to participate, they will be scheduled for an interview with transfusional medicine staff, once the quarantine time for Covid19 has been completed for the signing the informed consent. The selection criteria will be reviewed again and after the signing of the informed consent, screening studies will be carried out, including routine blood donation tests and PCR negative for Covid19.

The narration of the informed consent process must be registered in the donors electronic/paper medical record in a contemporary way. The following information will be recorded: date the informed consent was given to the patient for reading, name of the doctors who attend the informed consent process, start time, signature time, questions asked by the donor and their answers , version of the informed consent that was used, proof that the patient keeped an original informed consent form signed by one of the doctors delegated for such function. The medical team will keep an original signed by the patient, with verification of any condition of individual vulnerability of the patient according to resolution 1480/11 of the Ministerio de Salud de la República Argentina.

5.2.2 Patients not hospitalized at the time of Informed Consent

Patients with a positive RT-PCR for SARS-CoV-2, who have not been hospitalized or who have been hospitalized at another institution and who wish to donate plasma will be able to participate in this protocol as described below.

The information sheet and informed consent for donors will be sent to the potential donor by email and a telephone interview will be arranged. During the interview, the researcher will explain the protocol and the details of the donor's participation in it. All the doubts that the potential donor may have will be answered and if they express the will to donate, they will be summoned to the transfusional medicine service according to the eligibility criteria indicated in the selection criteria for donor part of this protocol. The narration on the date on which the documents and details of the telephone conversation were sent will be recorded in the donor´s medical record.

The transfusional medicine service staff will comply with all institutional biosafety measures in the context of Covid-19 for the care of donors. When attending to the transfusional medicine service, the donor will sign together with the delegated physician for this purpose two copies of informed consent; one original will be for the donor and the other for the center. The original

will be kept in an envelope pending the PCR result for COVID-19.

5.2.3 Patients who have been discharged at the time of Informed Consent

Patients who have already been discharged will be contacted by telephone by a researcher to inform them about the possibility of participating in the protocol through plasma donation.

In case they are interested in participating in it as volunteers, the information sheet and informed consent for donors will be sent by email and a telephone interview will be arranged. During the interview, the researcher will explain the protocol and the details of the donor's participation in it. All the doubts that the potential donor may have, will be answered and if they express the will to donate, they will be summoned to the hemotherapy service according to the times indicated in the selection criteria for making the donation. The narration on the date on which the documents and details of the telephone conversation were sent will be recorded in the donor´s medical record.

The transfusional medicine service staff will comply with all institutional biosafety measures in the context of Covid-19 for the care of donors. When attending the hemotherapy service, the donor will sign together with the delegated physician for this purpose two copies of informed consent; one original will be for the donor and the other for the center. The original will be kept in an envelope pending the PCR result for COVID-19.

6. Safety

6.1 Evaluation and recording of Adverse Events (AEs) and Adverse Drug Reactions (ADRs).

An adverse event is: any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An adverse drug reaction is defined as any unintended, harmful response to a drug that occurs at doses customary for prophylaxis, diagnosis, and treatment.

The researcher will evaluate each Adverse Event: the type of event and description of the event, maximum intensity, duration, conduct taken, causal relationship with the medication under study and evolution.

6.1.1 Intensity

1. Mild: awareness of signs or symptoms but easily tolerated.
2. Moderate: sufficient discomfort to cause interference with usual activity.
3. Severe: disabling, unable to work or perform usual activity.

6.1.2 Duration

Registration of the day and time of start and ending of the adverse event. If the duration was less than one day, the length of time will be recorded in the corresponding units.

6.1.3 Action taken with study medication

Record of action taken regarding plasma/placebo transfusion:
1. Plasma / placebo administration was continued.
2. Plasma / placebo administration was permanently suspended.
3. Plasma / placebo administration was temporarily suspended.
4. The study was suspended by the investigator.

5. The subject was withdrawn from the study.
6. The study was suspended by the sponsor.
7. None
In addition to the description of the conduct taken with respect to the study medication, it should be noted whether the patient required any specific treatment for the event.

6.1.4 Causal relationship with the medications under study

1. Definitive: Definitely related to the study medication
- There is evidence of exposure.
- The time sequence between the beginning of the adverse experience and the administration of the study medication is reasonable.
- The adverse experience is more likely explained by the study medication than by another cause.
- Positive re-exposure (if feasible).
- Adverse experience shows a pattern consistent with prior knowledge of the study medication or its class of drug.

2. PROBABLE: Probably related to study medication:
- There is evidence of exposure.
- The time sequence between the beginning of the adverse experience and the administration of the study medication is reasonable.
- The adverse experience is more likely explained by the study medication than by another cause.
- Re-exposure not carried out or positive if carried out.
- Adverse experience may or may not show a pattern consistent with prior knowledge of the study medication or its class of drug.

3. POSSIBLE: Possibly related to study medication
- There is evidence of exposure.
- The time sequence between the beginning of the adverse experience and the administration of the study medication is reasonable.
- The adverse experience could have been caused by another cause with equal probability.
- Positive re-exposure (if carried out).
- Re-exposure not carried out.
- Adverse experience may or may not show a pattern consistent with prior knowledge of the study medication or its class of drug.

4. NOT RELATED: not related to study medication
- There is evidence of exposure.
- There is another more probable cause of the adverse experience (ex: underlying disease)
- Negative or ambiguous re-exposure (if carried out).

5. NOT EVALUABLE
- It is not possible to analyze the causal relationship between the intervention and the adverse event.

6.1.5 Evolution

1. Continues
2. Recovered with sequel
3. Recovered without sequel
4. Deceased
5. Unknown

Taking into account this evaluation, the investigator must report the Adverse Event in the subject's Medical Record and will be responsible for reporting Serious Adverse Events to the ethics committee in a timely manner.

6.2 Serious Adverse Event (SAE) or Serious Adverse Reaction.

A Serious Adverse Event (SAE) is any unfavorable medical reaction or occurrence, at any dose level of a study medication, that occurs during the time the subject receives the study medication or within 30 days after the transfusion that:

1. Results in death.
2. It involves risk to life. It refers to an event in which the subject was at risk of death at the time the event occurred. It does not refer to an event that hypothetically would have caused death if it had been more severe.
3. Requires hospitalization or prolongation of hospitalization.
4. Results in significant or persistent disability or incapacity.
5. It implies a malformation or a congenital defect.
6. Clinically significant: not immediately life-threatening, not resulting in death or hospitalization, but the subject requires intervention to prevent other outcomes

If it is suspected that it presents some degree of causal relationship with the administration of the drug, it can be considered a Serious Adverse Drug Reaction. Any Serious Adverse Drug

Reaction that differs in nature or severity from the information known so far about the research product will be considered unexpected (SUSAR).

6.3 Serious Adverse Events (SAEs) management procedure

In the occurrence of a SAEs, the researcher must be contacted immediately by the center's staff, by the subject (interned or discharged) or by the center's guard service, if applicable (if it occurs after discharge from the center).

The investigator must report the SAE in the Clinical History and complete the CRF sheet corresponding to ADVERSE EVENTS and the one for SERIOUS ADVERSE EVENTS. The investigator must inform the institutional ethics committee in a timely manner.

If the information is initially limited, additional information must be sent later. The researcher must ensure that the information obtained by telephone or by other means reported in the Clinical History and CRF is accurate and consistent with reality.

The researcher must carry out the pertinent follow-up of the SAE, until its resolution, including that belonging to a subject withdrawn (discontinued) from the study.

6.4 Abnormal results of laboratory analysis.

The results of all the laboratory tests performed subjects will be attached to the CRFs of each subject; sensitive data will be deleted from them.

After plasma / placebo transfusion, a laboratory parameter with a clinically significant abnormal result should be recorded in the subject's Medical Record and in the CRF as Adverse Event or Serious Adverse Event, as appropriate.

Abnormal laboratory values will be followed to provide the best possible medical care.

6.5 Criteria for stopping treatment during transfusion

Any adverse event that occurs during the transfusion will be evaluated by the treating physician and, according to the severity and safety evaluations, will decide whether or not to continue with the study.

The occurrence of anaphylaxis, which is 8 per 100,000 transfused units-UT, is defined as: acute inflammatory reaction resulting from the release of histamine and histamine-like substances by mast cells that trigger a response immune from hypersensitivity that can manifest clinically with respiratory distress, bronchospasm, dizziness, hypotension, cyanosis, loss of consciousness, edema / angioedema with or without urticaria. If the research subject presents any sign or symptom compatible with anaphylaxis he/she will be treated in a timely manner according to the Emergency protocols of Hospital Italiano. The plasma infusion will be suspended and the risk / benefit ratio of trying to transfuse it again with greater safety measures and eventually premedication will be evaluated. The subject will be monitored and followed by the Principal Investigator until the event is resolved.

Other events of very low incidence that may motivate the suspension of the transfusion: i) acute worsening of the hemodynamic and / or respiratory status caused by circulatory overload associated with transfusion (TACO); ii) respiratory and / or hemodynamic deterioration caused by transfusion-associated acute lung injury (TRALI).

TRALI is defined as an entity characterized by the development, within 6 hours of transfusion, of non-cardiogenic pulmonary edema due to endothelial damage caused by the interaction of antibodies against human lymphocytes (HLA) or against human neutrophils (HNA), or of inflammatory mediators with the pulmonary endothelium: risk factors for TRALI are: liver surgery, chronic alcohol abuse, high peak pressure in the airway during mechanical ventilation, smoking, positive fluid balance and elevated levels of Il-8 (0 , 4/100000 TU). To reduce the risk of this entity, multiparous women are excluded as donors.
Finally, transfusion-associated circulatory overload (TACO) affects, according to some studies, between 1 and 8% of transfused patients or approximately 1 in 9,177 TU. The picture consists of the onset or worsening of respiratory distress within 4-6 hours of the transfusion, elevated brain natriuretic peptide (BNP or NT-pro-BNP), increased central venous pressure, left heart failure, positive balance fluid or pulmonary edema.

6.6 Planned rescue treatment and follow-up in cases of failure or adverse events.
During the administration of plasma, the patient will be admitted to the institutional Intensive Care Unit if necessary. You will be monitored during your hospitalization by a doctor and a nurse designated exclusively for this task. In an emergency, we will proceed according to the institution's validated emergency management protocols. The patient will be cared for, giving him life support and adequate treatment. If necessary, he will be transferred to the Intensive Care Unit.

7. Ethical and regulatory considerations

All participating centers will present the project for evaluation and eventual approval by the corresponding institutional Ethics Committees. In accordance with the regulations established by the Strategic Plan to regulate the use of plasma from patients recovered from Covid-19 for therapeutic purposes defined by the Secretariat of Quality in Health of the Nation on April 16, this project will be informed to the National Directorate of Hemoderivatives of the Ministry of Health of the Nation.

Plasma donors must give their informed consent for this purpose.

The patients to be treated will also require the corresponding Informed Consent process completed with the signing of the document as a step prior to the incorporation of all patients who express their willingness to participate in the clinical trial.

Once completed, the Database of patients enrolled in the clinical trial will be registered with the National Directorate for the Protection of Personal Data, as well as the considerations about maintaining the confidentiality of the data incorporated into the text of the Informed Consent forms. They are framed within the directives established by Law 25.326 on the Protection of Personal Data. The present project as a whole will be carried out within the framework established by the Declaration of Helsinki, resolution 783/2020 (Strategic Plan to Regulate the Use of Plasma by Patients Recovered from COVID-19 for Therapeutic Purposes) and resolution 1480 / 11 of the MSN and resolution 1480/11 of the Ministry of Health of the Nation in relation to research on human beings.

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Part I. B:
Study Protocol (version 3.0)

Convalescent plasma in patients with Covid-19 severe pneumonia; a multicenter, randomized, placebo controlled clinical trial. The PlasmAr study

1. Introduction

Since spanish flu in 1918, convalescent plasma has been used for the treatment of some infections with the assumption that passive immunization can provide the amount of neutralizing antibodies necessary to control the evolution of the disease while establishing the patient's immune response [1]. This strategy was also used more recently in treating the severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), influenza A (H1N1) in 2009, avian influenza (H5N1) and Ebola, with different results.

There is previous experience in the use of convalescent plasma in patients with Argentine Hemorrhagic Fever (ALF) in our country. In a double-blind study carried out by the Institute of Virology of Pergamino between 1974 and 1977, which included 217, and 188 were finally randomized, there was a significant decrease in mortality [2]. Among the conclusions it is worth highlighting that the administration within 8 days of the onset of symptoms as a determining factor.

Table 1 shows the primary outcome from the Argentine Hemorrhagic Fever study [2]

TABLE I—MORTALITY IN PATIENTS WITH AHF TREATED WITH IMMUNE OR NORMAL PLASMA

Treatment	Total cases	Improved	Died	Mortality (%)
Immune plasma	91	90	1	1·1
Normal plasma	97	81	16	16·5
Total	188	171	17	—

χ_1^2=13·53; p<0·01

At present, given the rapid expansion of the epidemic of SARS by coronavirus 2 (SARS-CoV-2) known as Covid-19, different scientific groups and government authorities are working on different measures and regulations that favor the implementation of experimental treatments based on pathophysiological mechanisms or supported by low quality evidence [3].

In March 2020, a first report from *Shen et al*. based on the experience in five patients with COVID-19 treated successfully with convalescent plasma, raised worldwide expectations regarding its clinical role in fighting this new disease [4,5]. However, an editorial in the same journal raised important arguments about the real efficacy of this therapeutic strategy, mentioning that, although the cases reported by *Shen et al*. were adequately presented and well studied, the research had important limitations, highlighting that it was not possible to

determine the real clinical effect of the plasma intervention and / or ensure that the patients had recovered as a result of this intervention, since other therapeutic options such as corticosteroids and antivirals had also been applied [5]. Another controversial point would be the ideal time for the administration of convalescent plasma. In this case, the plasma infusion was carried out 3 weeks after hospital admission, and its effect on earlier administration was not clear [45].

Another study that provides evidence on the use of convalescent plasma and the timing of its administration is the non-randomized study presented by Cheng et al in 2005. Reinforcing what was reported by Maiztegui et al, the study showed that the time of plasma administration was statistically different between the groups, giving a relevant role to this variable. Age and specific antibody development were other statistically important variables influencing prognosis of these patients. Patients who had a positive reverse-transcriptase-polymerase-chain-reaction (RT-PCR) and negative coronavirus serology at the time of convalescent plasma administration, had better outcomes than those who were already seropositive (66.7% vs 20% p = 0.001). In the multivariate analysis, only the convalescent plasma administration time and the positive RT-PCR were statistically significant [6].

2. Study Proposal

2.1 Study proposal and hypothesis

General purpose of the study: to test the efficacy and safety of convalescent plasma for the treatment of Covid19 pneumonia

Study Hypothesis: Convalescent Plasma significantly improves outcome of severe Covid-19 pneumonia in patients

2.2 Study design

The PlasmAr study is a multicenter, double-blind, placebo-controlled. The randomization will be asymmetric 2:1.

2.2.1 Rational basis of the intervention

There is no specific therapy of proven effectiveness against Covid-19. Several therapeutics lines are currently being studied internationally. In this context, the administration of antibodies through the transfusion of convalescent plasma appears as a reasonable option for research. This strategy has already been recommended as empirical treatment during the Ebola and MERS epidemics[4], and within the context of clinical research in severe influenza illness. Recently, a series of uncontrolled cases of patients with Covid-19 who improved their clinical condition after the administration of convalescent plasma was published. Currently, several clinical studies with convalescent plasma are being evaluated or already implemented in different regions of the world [56].

2.2.2 Rationale for the use of Placebo control arm

The specific effectiveness of the use of convalescent plasma in the treatment of Covid-19 pneumonia is not known. Although the case series and reports offer encouraging results, there is no high-quality evidence on this regard. In addition, the methodology for plasma collection, administration and control is relatively complex and its efficacy should be convincingly established before promoting it for healthcare use.

Controlled clinical trials of the use of hyperimmune immunoglobulin in patients with severe influenza did not confirm the results that previous observational studies had suggested. The overall risk of pool plasma transfusion of patients is very low. Beyond this, it has been clearly recommended to prioritize the application of therapeutic strategies in the context of clinical studies over the empirical use of treatments with as yet unproven efficacy [78] .

In the case of the present study, the intervention strategy is proposed in an “add on” modality, as eventual antiviral treatment already been initiated, may not preclude the addition of

convalescent plasma, since it is a completely different type of therapeutic approach. For this reason, the participation of patients in the present study will not condition their opportunity to receive other types of treatments, both in the plasma and in the placebo arms.

2.3 Study population

Patients with a confirmed diagnosis of Covid-19 pneumonia hospitalized in any of the participating institutions, who present the inclusion criteria of the study and voluntarily provide their informed consent.

2.4 Scope of the study and participating institutions

La multicenter clinical trial will be carried out at the Hospital Italiano de Buenos Aires and other centers in Ciudad Autónoma de Buenos Aires, Buenos Aires province and other provinces.

2.5 Study aims

2.5.1 Primary outcomes

We will analyse the difference in patient's clinical status day 30 after intervention, represented by one of the following six mutually exclusive ordinal categories [9]:
1- death
2- invasive ventilatory support
3- hospitalized with supplemental oxygen requirements
4- hospitalized without supplemental oxygen requirements
5- discharged without full return of baseline physical function
6- discharged with full return of baseline physical function

2.5.2 Secondary outcomes

1. 6 categories ordinal outcome on day 7th after intervention.
2. 6 categories ordinal outcome on day 14th after intervention.
3. Time from intervention to discharge from hospital (in days).
4. Time from intervention to discharge from the ICU (in days).
5. Time from the intervention to complete restitution of physical functions (according to baseline status).
6. Time from the intervention to the start of invasive ventilatory support.
7. Time from intervention to death (in days).
8. Time to improvement of 2 categories in the ordinal outcome or hospital discharge within 30 days.

9. Percentage of participants with adverse events / serious adverse events.
10. Serum D-dimer (ng/ml) plasma concentration at day 14.
11. Serum Ferritin (ng/ml) plasma concentration at day 14.
12. Plasma concentration of total antibodies on day 2 after the intervention.
13. Plasma concentration of total antibodies on day 7 after the intervention.
14. Percentage of post-transfusion specific adverse reactions between groups.

2.6 Inclusion/exclusion criteria of the patients

To enter the study, patients must meet the inclusion criteria and none of the exclusion criteria. Patients who can potentially be admitted to the study will receive the usual treatments and may be receiving other specific treatments for COVID-19

2.6.1 Inclusion criterias

- Patients older than 18 years
- Confirmed diagnosis of Covid-19 by qualitative reverse transcriptase-polymerase chain reaction (qRT-PCR -GeneDX Co, Ltd or similar).
- Diagnosis of pneumonia by imaging (Rx or CT).
- One or more of the following criteria:
    - Score MSOFA (Modified SOFA) [10] or conventional SOFA with a score of 2 or higher, or a difference of at least +2 points from baseline for patients with a baseline score other than 0 (modified sequential evaluation criteria for organ failure)
    - Resting oxygen saturation less than 93% breathing at room air
    - PaFi (Ratio of arterial oxygen pressure over inspired fraction of oxygen) less than 300
- Provision of informed consent by the participant

Table 2: MSofa score [10]

Modified Sepsis-related Organ Failure Assessments (MSOFA)

Score	0	1	2	3	4
Respiration PaO_2(mmHg) /FiO_2 SPO_2/FiO_2 (%)	>400 >400	<400 <400	<300 ≤ 315	<200 ≤ 235 With Respiratory support	<100 ≤ 150 With Respiratory support
Liver	No scleral icterus or jaundice			Scleral icterus or jaundice	
Cardiovascular Hypotension *ug/kg/min for 1 hour	MAP≥70	MAP <70	*Dopamine <5 or *Dobutamine (any dose)	*Dopamine5.1-15 or *Adrenaline≤0.1 *Norepinephrine≤0.1	*Dopamine>15 or *Adrenaline>0.1 *Norepinephrine>0.1
CNS Glasgow Coma Scale	15	13-14	10-12	6-9	<6
Renal Creatinine (mg/dL) Creatinine (umol/L) Urine Output (mL/day)	<1.2 <106	1.2-1.9 106-170	2.0-3.4 177-301	3.5-4.9 309-433 <500	>4.9 >433 <200
MSOFA mortality: 0-7=4%; 8-11=31% ; ≥11=58%			**SOFA mortality: 0-7=5%; 8-11=28% ; ≥11=53%**		

Adapted from Grissom, CK, Brown, SM, Kuttler, KG, et alA modified sequential organ failure assessment score for critical care triage. *Disaster Med Public Health Prep.* 2010;4 (4):277–284

Prepared by Malaysia Sepsis Alliance (MySepsis) & Universiti Kebangsaan Malaysia Medical Center. Link: www.mysepsis.org

2.6.2 Exclusion criterias

- Pregnancy
- People of reproductive age who do not agree to avoid having unprotected sex until day 30 after the start of the study
- Breastfeeding women
- Patients who have received experimental treatments under development within 30 days prior to admission
- Patients with a history of allergic reactions to blood transfusions or its components
- Confirmation of another concomitant microbiological cause of pneumonia other than Covid-19
- Patients who are on mechanical ventilation, with multiple organ failure or who for any other reason cannot voluntarily give their consent.
- Patient who is not going to progress the treatment at the discretion of the treating physician
- Patients who after more than 24 hs of being offered the trial, decides to participate, provided that he/she does not meet an exclusion criterion in that period.

2.7 Clinical procedures

The patients will be selected by physicians of intensive care units, intermediate care units and infectious diseases department.

The Informed Consent process will be carried out in accordance with the provisions of point 5 of this protocol.

The investigator will again review the inclusion / exclusion criteria of the patients based on data from the physical examination and laboratory parameters.

Then the patient will be randomized.

The following table details the schedule of procedures to be performed on each patient included in the study.

Table 3: clinical procedures schedule

Procedures	**day 0**	**day 1**	**day 2**	**day 3**	**day 4-6**	**day 7**	**day 8-13**	**day 14**	**day 15-29**	**day 30**
Inclusion/exclusion criteria	X									
SARS-CoV-2 RT-PCR	X									
Informed consent	X									
Physical exam	X	X	X	X	X	X	X	X	X	X
Body mass index	X									
Heart/respiratory rate	X	X	X	X	X	X	X	X	X	X
SpO2/Fio2	X	X	X	X	X	X	X	X	X	X
Temperature (C°)	X	X	X	X	X	X	X	X	X	X
MSofa score	X					X		X		
Intervention	X									
Primary outcome		X	X	X	X	X	X	X	X	X
Concomitant drugs	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X
Neutralizing antibody testing	X		X			X		X		
Total antibody testing	X		X			X		X		
Ferritin level	X							X		
D-dimer level	X							X		

Complete blood count exams	X			X		X		X		
Chemistry exams	X			X		X		X		
Pregnancy test	X									X

The procedures detailed in the protocol will be performed within the context of the hospitalization of participants. In addition to the procedures indicated by the protocol each of the participating centers will perform the procedures and analytical exams that they consider necessary for the best care of patients. In the event that the participant is discharged from the institution prior to the deadlines for the indicated visits in the schedule, the follow up will be performed by telephone, in the corresponding visits (7, 14 and 30). For interval visits (8-13 and 15-29), a minimum of two phone calls for retrieving safety data will be carried out.

If the patient cannot attend to collect the study analytical samples when appropriate or is not possible to take them at home, those missing samples will be documented in the medical record.

During all the development of the study but in particular in case of early hospital discharge, the need for all participants of childbearing age to maintain measures of effective contraception will be enforced.

2.7.1 Convalescent plasma safety issues

The safety assessment of the administration of plasma is described in point 6. Regarding the safety of plasma in terms of apheresis and safety measures, the recommendations of the Ministerio de Salud de la Nación will be followed and those in force by the accreditations of the Hospital Italiano de Buenos Aires as described in point 2.7.2

2.7.2 Convalescent plasma/placebo infusion: schedule and administration route

Once included in the study, the patient will be monitored and the infusion will begin. The plasma / placebo will be infused intravenously, preferably through one of the branches of a central vein catheter, if the patient had one in place to ensure complete infusion of the plasma / placebo. If not, it is recommended that it be the safest route available to the patient.

The infused volume will be defined within the range of 5-10 ml/kg with an inferior limit around 400 ml for patients whose body weight was below 70 kg and a superior limit of 600 ml for those above 70 kg. An administration rate of 5-10 ml/kg/h is recommended, although the final rate

could be adapted in accordance with the patient's tolerance and/or risk of volume overload. No standardized premedication is required before transfusion. Patients must be clinically monitored throughout the entire transfusion process in order to assist and register any incidental adverse reaction.

2.7.3 Patient follow up

During the plasma / placebo infusion, vital signs and symptoms of adverse reactions, including acute hypersensitivity reactions, should be monitored. In the event of an emergency, we will proceed according to the emergency management protocols validated in each institution. The patient will be cared for, giving him life support and adequate treatment.

2.8 Analysis plan and statistical methodology

2.8.1 Randomization

The randomization process will consist of two stages: randomization and allocation concealment.

For the random assignment, a random list will be generated in blocks of variable size, stratified for each of the participating centers. Recruitment will be consecutive until the proposed sample size is reached. The randomization will be done from RedCap with non-blind users belonging to the Hemotherapy Service who will be the only ones authorized in this procedure.

A list of random numbers will be drawn up for each of the participating centers, consigning the assignment to the branch "Intervention" (Convalescent Plasma) or "Placebo" (saline solution).

This list will remain hidden from both the doctors and nurses in charge of the assistance of each participant. The result of the assignment will be reported from the central protocol coordination structure to the head of Transfusion Medicine of each of the institutions. Those responsible for Transfusion Medicine or Hemotherapy of each center will prepare the container containing the plasma or placebo, as appropriate. Since the plasma solution is slightly opaque, the container and IV line will be covered with a colored sleeve to maintain the masking.

The statistical team and the person in charge of transfusional medicine will be unblinded to intervention. The rest of the researchers will remain blinded to the intervention branches of the study.

2.8.2 Statistical analysis

2.8.2.1 Sample size calculation

It was assumed that 52% of patients in the control arm would be in categories 6 or 5 at 30 days compared to 66% in the CP group. To achieve 80% power to detect a proportional odds ratio between arms of at least 1.8 in the clinical ordinal scale at the 0.05 (2-sided) level of significance, a sample size of 333 patients (222 in plasma arm and 111 in placebo arm) was defined. Odds ratio greater than 1.0 correspond to more favourable outcomes on CP use compared with placebo.

2.8.2.2 Statistical methodology

We included all randomized patients in the analysis according to the randomization arm (intention-to-treat analysis). We anticipate that all patients will receive the once time intervention, but if this does not occur, the possibility of performing a per-protocol analysis is contemplated. In all cases we used the total number of participants who contributed values. Categorical variables will be presented as absolute and relative frequency in percentage. Continuous variables will be summarized as mean and standard deviation (sd) or median and interquartile interval (*IQR*) according to the observed distribution. We will apply logarithmic transformations for continuous variables with asymmetric distribution. We will apply the Wilcoxon rank sum test to compare the distribution of continuous and logarithmic transformed variables between exposure arms.

We will evaluate the association between convalescent plasma or placebo and the ordinal primary outcome, using an ordinal logistic regression model . We will use this model to estimate a common proportional odds ratio (OR) for the ordinal categories of the primary outcome between arms on the 7th, 14th and 30th day [1111,12]. The proportional odds ratio assumption is evaluated using the Brant parallel regression assumption test [1313,14].

For time to combine events including improvement of 2 categories in the ordinal outcome or hospital discharge within 30 days, we will consider deaths within 30 days, as censored at day 30 as a different approach to consider death as a competing event. We will use Cox proportional hazard regression model to evaluate time to death and time to clinical improvement and to estimate the Hazard Ratios (HR). We will use the Kaplan Meier method to estimate the cumulative incidence as a function of time. We will use Fine and Gray regression models considering death as a competing event to estimate the subHazard Ratios (sHR) for the association between arm of exposure and time to discharge from hospital, discharge from the ICU, complete restitution of physical functions, and start of invasive ventilatory support. We will use logistic regression models to estimate the OR for the comparison of adverse events

between arms. All association measures will be presented with 95% confidence intervals (95%CI).

Planned subgroup analysis will be performed according to: age groups, gender, time/delay from onset of symptoms to intervention, presence of comorbidities (chronic obstructive pulmonary disease, obesity, immunosuppression, diabetes, hypertension, cardiovascular disease), baseline participant antibody titer and recruiting sites and corticosteroid concomitant treatment. We will present the interaction test *p* values with the estimated OR for each stratum. For comparisons of secondary outcomes, we will consider statistically significant p values of less than 0.05.

3. Study Intervention

3.1 Technical procedures: plasma preparation

3.1.1 Identification and recruitment of potential donors

- The identification of plasma donors may be as follows:

  - Patients hospitalized for COVID-19 will be invited to donate plasma after recovery and when they meet the inclusion criteria.

  - A list of potential donors will be obtained from the registry of convalescent patients.

  - Contact convalescent patients by phone

- The PCR requirement for SARS-CoV-19 for donors will be adapted to the official regulations in force at the time of donation.
- Informed consent process
- Complete the "blood and / or blood component donor eligibility" questionnaire
- Prior to donating plasma, the donor will be asked to collect samples for pre-donor studies, serology, molecular biology (HIV-HBV-HCV) and immunohematology of apheresis pre-donor samples to titrate specific antibodies by ELISA .
- Based on the specific antibody titer, your eligibility will be determined.
- In order to guarantee traceability, they will be incorporated into a system that ensures traceability in each participating center.
- On the day of extraction, a volume that does not exceed 15% of total blood volume will be obtained from each donor. In the absence of a plasmapheresis equipment, manual plasmapheresis can be performed.
- Units of convalescent plasma will be identified with a label that clearly identifies these

units.

- The plasma will be stored at 4 degrees until the infusion, if the infusion is carried out within 48 hours of its collection
- The plasma will be frozen at -80 degrees and stored at -40 degrees until the infusion, if the infusion is carried out after 48 hours from its collection
- The place of storage will be in a specific compartment identified as “Convalescent Plasma SARS-CoV -2 "

3.1.2 Inclusion criteria for plasma donors

- General acceptance criteria for blood donors according to Administrative and Technical Regulations RM 797/13 - 139/14 - 1507/15. Directorate of Blood and Hemoderivatives of the Ministry of Health of the Nation, Argentine Association of Hemotherapy, Immunohematology and Cell Therapy (AAHITC).
- Age: 18 to 60 years.
- People who have recovered from the SARS-CoV-2 infection
- Previously diagnosed for COVID-19 and who comply with the official regulations in force at the time of donation regarding PCR.
- The donor must complete a period of 28 days for complete resolution of symptoms.
- Multiparous donors must be negative for anti-HLA antibodies. If the determination of anti-HLA antibodies cannot be carried out, multiparous donors will not be accepted.
- The specific titer of total antibodies should be> 1/400
- Study profile of transfusion transmissible infections (TTI) must be negative for hepatitis B virus, hepatitis C virus, HIV, syphilis, brucellosis, HTLV and Chagas.
- The donor must read, understand and voluntarily sign the informed consent for Apheresis Plasma Donation.

An immunohematological profile of the donated blood will be carried out according to standard practice (ABO/Detection of Irregular Antibodies/quantitative determination of Isohemagglutinins). The blood group of all recipients will be requested to analyze compatibility with the plasma pool.

Between 400 and 600 mL of plasma will be obtained from each donor using Continuous Flow and/or discontinuous cell separators validated and approved by ANMAT (Ex: Trima Accel, Terumo, Spectra optia Terumo, COMTEC), or through validated manual procedures that comply with the required standards of traceability and biosafety adjusting to current regulations.

These inclusion criteria are taken to minimize the risk of administering COVID19-infected

plasma to patients, despite the fact that there is literature that ensures that the use of plasma with possible infection would not be adding burden to patients with a diagnosis.

3.1.3 Convalescent plasma

- Caption on Convalescent Plasma container: Caution. New drug: limited to investigational use.
- Plasma pools of up to 5 donors will be made according to the regulations in force in order to homogenize the intervention.
- Plasma pools can be used regardless of compatibility group, if the anti A and / or Anti B antibodies present a titer no greater than 1/64 according to the guidelines of the Argentine Association of Immunohematology Hemotherapy and Cellular therapy.
- If a pool has Anti A titer> 1/64, it can only be transfused in patients of group O and B according to the clinical practice guidelines of the Argentine Association of Hemotherapy Immunohematology and Cell Therapy.
- If a pool presents Anti B titer> 1/64, it can only be transfused in patients with group O and A according to the clinical practice guidelines of the Argentine Association of Hemotherapy Immunohematology and Cell Therapy.

The plasma will be labeled as COVID-19 Convalescent Plasma appearing as directed in the traceability system for designated recipient

3.2 Methodology for the analysis of total and neutralizing antibodies

The commercial laboratory kit that has the approval of regulatory agencies for the dosage of total antibodies will be used for this project. These commercial kits will also be validated in the central laboratory of the Hospital Italiano de Buenos Aires. Once these kits have been validated, they can be used by all the centers participating in the present study.

Regarding neutralizing antibodies, it will be carried out at the Leloir Institute (Dr. Andrea Gamarnik Laboratory).

3.3 Neutralizing antibody dosing calculation

A pool will be made with plasma donations and the total antibody titer will be measured to ensure minimum antibody titers.

Measurement of neutralizing antibodies will be performed as soon as the test is available and validated. For this purpose, an aliquot of plasma from each patient will only be frozen for future measurement.

In addition, prior to the study infusion, an aliquot of the necessary sample to test blood group and factor will also be kept for the measurement of total antibodies and neutralizers of the patients.

There is no information on what is the minimum neutralizing antibody titer for this disease.

It will ensure that the patient receives a plasma transfusion that has at least a total antibody titer of 1: 1000.

3.3.1 Infusion product: convalescent plasma/placebo

- The plasma will be labeled Convalescent Plasma / COVID-19 Placebo as directed in the designated recipient traceability system.
- To maintain the masking of the study, both the bag and the tubing will be covered.
- Plasma will be infused immediately to patients who meet the inclusion criteria for this treatment.
- If necessary, the plasma can be frozen as Fresh Frozen Plasma.

3.4 Procedures for unmasking assignment

The intervention will remain blind to the research subjects and to the research team throughout the entire protocol. The transfusional medicine team and the persons in charge of the statistical analysis will not be blind regarding the assignment of the branch to which the patients have been randomized.

Opening of the blinding will only be contemplated in the event that a patient dramatically worsens clinical condition, and the treating physician considers that the patient could benefit from a plasma transfusion. The administration of any other specific treatment (other than plasma) does not require opening of the blinding since this protocol allows other concomitant therapies for the treatment of Covid-19.

If unmasking is asked, a Clinical Evaluation Committee may be consulted, which will define the relevance of opening the blind. This Committee will be made up of three physicians designated for this purpose. The case will be analyzed and the committee's recommendation will not be binding but will give the treating physician support to better consider the case. The doctor must ask the transfusional medicine team to open the blind to know the branch to which the patient was assigned.

The assessment of the patient will take into account the treatments established, the possibility and access to other treatments, comorbidities and the general condition of the patient.

The analysis that will be given to the data of patients whose blind has been opened is detailed in the statistical analysis section.

4. Case report form and schedule of activities

The epidemiological, demographic, clinical, laboratory, treatment and results data will be extracted from the clinical history of each patient. The data will be collected using a standardized data collection form which will be implemented electronically through the RedCap® platform.

5. Informed consent form

All patients included in the study must give their consent as well as plasma donors. Both models of Informed Consent Forms are attached. 5.1

5.1 Procedures for informed consent in participants

5.1.1 Procedures for informed consent in included patients

All patients enrolled in the study will be hospitalized with an indication for isolation due to their pathology. The consent process can be adapted to the conditions of each of the centers. The consent process will be carried out according to the following steps:

- Once the patient is identified, the doctor delegated to take consent will sign it, putting the time at which it is done.
- The delegate physician will deliver to the assistance personnel who are in possession of a Personal Protective Equipment (PPE) for their task and they will deliver it to the patient. After having read the informed consent, if the patient has any questions, they can make a phone call to the doctor who signed the consent (these patients usually have their personal phone in their possession). The patient signs the consent after the explanations (he/she will put the time at which he signs).
- Once the consent is signed, it is considered contaminated and will be placed in a plastic bag. This process will take place in the patient's room. The assistance personnel who put the consent on the bag, will put it in a paper envelope that will be held by the person who is outside the room. This envelope will be considered clean.
- In this paper envelope, the initials of the patient and the date on which it was consented will be labeled. Later it will be filed and unarchived 7 days after the signature.
- The entire process will be described in the patient's medical record in a contemporary way.
- In the event that the patient is considered vulnerable by the researcher or the doctor delegated to take consent, a witness will be added to the video call, who will be asked to sign the consent later. Details regarding Who the person was, what links them to the patient, their identification number and the phone from which they call will be recorded in the medical record when the process is described.

5.2 Procedures for informed consent in plasma donors

5.2.1 Patients who have NOT participated in the PlasmAr study prior to discharge

Patients who meet the selection criteria for a convalescent plasma donor will be invited to participate in the study, according to previously established procedures. They will be given the information sheet and the informed consent so that they can read it with confidence. Then they will be contacted by phone by doctors from the research team delegated for the taking of informed consent to talk about the procedure and evacuate the donor's doubts.

If the potential donors are willing to participate, they will be scheduled for an interview with transfusional medicine staff, once the quarantine time for Covid19 has been completed for the signing the informed consent. The selection criteria will be reviewed again and after the signing of the informed consent, screening studies will be carried out, including routine blood donation tests.

The narration of the informed consent process must be registered in the donors electronic/paper medical record in a contemporary way. The following information will be recorded: date the informed consent was given to the patient for reading, name of the doctors who attend the informed consent process, start time, signature time, questions asked by the donor and their answers , version of the informed consent that was used, proof that the patient keeped an original informed consent form signed by one of the doctors delegated for such function. The medical team will keep an original signed by the patient, with verification of any condition of individual vulnerability of the patient according to resolution 1480/11 of the Ministerio de Salud de la República Argentina.

5.2.2 Patients not hospitalized at the time of Informed Consent

Patients with a positive RT-PCR for SARS-CoV-2, who have not been hospitalized or who have been hospitalized at another institution and who wish to donate plasma will be able to participate in this protocol as described below.

The information sheet and informed consent for donors will be sent to the potential donor by email and a telephone interview will be arranged. During the interview, the researcher will explain the protocol and the details of the donor's participation in it. All the doubts that the potential donor may have will be answered and if they express the will to donate, they will be summoned to the transfusional medicine service according to the eligibility criteria indicated in the selection criteria for donor part of this protocol. The narration on the date on which the documents and details of the telephone conversation were sent will be recorded in the donor´s medical record.

The transfusional medicine service staff will comply with all institutional biosafety measures in the context of Covid-19 for the care of donors. When attending to the transfusional medicine service, the donor will sign together with the delegated physician for this purpose two copies of informed consent; one original will be for the donor and the other for the center.

5.2.3 Patients who have been discharged at the time of Informed Consent

Patients who have already been discharged will be contacted by telephone by a researcher to inform them about the possibility of participating in the protocol through plasma donation.

In case they are interested in participating in it as volunteers, the information sheet and informed consent for donors will be sent by email and a telephone interview will be arranged. During the interview, the researcher will explain the protocol and the details of the donor's participation in it. All the doubts that the potential donor may have, will be answered and if they express the will to donate, they will be summoned to the hemotherapy service according to the times indicated in the selection criteria for making the donation. The narration on the date on which the documents and details of the telephone conversation were sent will be recorded in the donor´s medical record.

The transfusional medicine service staff will comply with all institutional biosafety measures in the context of Covid-19 for the care of donors. When attending the hemotherapy service, the donor will sign together with the delegated physician for this purpose two copies of informed consent; one original will be for the donor and the other for the center.

6. Safety

6.1 Evaluation and recording of Adverse Events (AEs) and Adverse Drug Reactions (ADRs).

An adverse event is: any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An adverse drug reaction is defined as any unintended, harmful response to a drug that occurs at doses customary for prophylaxis, diagnosis, and treatment.

The researcher will evaluate each Adverse Event: the type of event and description of the event, maximum intensity, duration, conduct taken, causal relationship with the medication under study and evolution.

6.1.1 Intensity

1. Mild: awareness of signs or symptoms but easily tolerated.
2. Moderate: sufficient discomfort to cause interference with usual activity.
3. Severe: disabling, unable to work or perform usual activity.

6.1.2 Duration

Registration of the day and time of start and ending of the adverse event. If the duration was less than one day, the length of time will be recorded in the corresponding units.

6.1.3 Action taken with study medication

Record of action taken regarding plasma/placebo transfusion:
1. Plasma / placebo administration was continued.
2. Plasma / placebo administration was permanently suspended.
3. Plasma / placebo administration was temporarily suspended.
4. The study was suspended by the investigator.

5. The subject was withdrawn from the study.
6. The study was suspended by the sponsor.
7. None
In addition to the description of the conduct taken with respect to the study medication, it should be noted whether the patient required any specific treatment for the event.

6.1.4 Causal relationship with the medications under study

1. Definitive: Definitely related to the study medication
- There is evidence of exposure.
- The time sequence between the beginning of the adverse experience and the administration of the study medication is reasonable.
- The adverse experience is more likely explained by the study medication than by another cause.
- Positive re-exposure (if feasible).
- Adverse experience shows a pattern consistent with prior knowledge of the study medication or its class of drug.

2. PROBABLE: Probably related to study medication:
- There is evidence of exposure.
- The time sequence between the beginning of the adverse experience and the administration of the study medication is reasonable.
- The adverse experience is more likely explained by the study medication than by another cause.
- Re-exposure not carried out or positive if carried out.
- Adverse experience may or may not show a pattern consistent with prior knowledge of the study medication or its class of drug.

3. POSSIBLE: Possibly related to study medication
- There is evidence of exposure.
- The time sequence between the beginning of the adverse experience and the administration of the study medication is reasonable.
- The adverse experience could have been caused by another cause with equal probability.
- Positive re-exposure (if carried out).
- Re-exposure not carried out.
- Adverse experience may or may not show a pattern consistent with prior knowledge of the study medication or its class of drug.

4. NOT RELATED: not related to study medication
- There is evidence of exposure.
- There is another more probable cause of the adverse experience (ex: underlying disease)
- Negative or ambiguous re-exposure (if carried out).

5. NOT EVALUABLE
- It is not possible to analyze the causal relationship between the intervention and the adverse event.

6.1.5 Evolution

1. Continues
2. Recovered with sequel
3. Recovered without sequel
4. Deceased
5. Unknown

Taking into account this evaluation, the investigator must report the Adverse Event in the subject's Medical Record and will be responsible for reporting Serious Adverse Events to the ethics committee in a timely manner.

6.2 Serious Adverse Event (SAE) or Serious Adverse Reaction.

A Serious Adverse Event (SAE) is any unfavorable medical reaction or occurrence, at any dose level of a study medication, that occurs during the time the subject receives the study medication or within 30 days after the transfusion that:

1. Results in death.
2. It involves risk to life. It refers to an event in which the subject was at risk of death at the time the event occurred. It does not refer to an event that hypothetically would have caused death if it had been more severe.
3. Requires hospitalization or prolongation of hospitalization.
4. Results in significant or persistent disability or incapacity.
5. It implies a malformation or a congenital defect.
6. Clinically significant: not immediately life-threatening, not resulting in death or hospitalization, but the subject requires intervention to prevent other outcomes

If it is suspected that it presents some degree of causal relationship with the administration of the drug, it can be considered a Serious Adverse Drug Reaction. Any Serious Adverse Drug

Reaction that differs in nature or severity from the information known so far about the research product will be considered unexpected (SUSAR).

6.3 Serious Adverse Events (SAEs) management procedure

In the occurrence of a SAEs, the researcher must be contacted immediately by the center's staff, by the subject (interned or discharged) or by the center's guard service, if applicable (if it occurs after discharge from the center).

The investigator must report the SAE in the Clinical History and complete the CRF sheet corresponding to ADVERSE EVENTS and the one for SERIOUS ADVERSE EVENTS. The investigator must inform the institutional ethics committee in a timely manner.

If the information is initially limited, additional information must be sent later. The researcher must ensure that the information obtained by telephone or by other means reported in the Clinical History and CRF is accurate and consistent with reality.

The researcher must carry out the pertinent follow-up of the SAE, until its resolution, including that belonging to a subject withdrawn (discontinued) from the study.

6.4 Abnormal results of laboratory analysis.

The results of all the laboratory tests performed subjects will be attached to the CRFs of each subject; sensitive data will be deleted from them.

After plasma / placebo transfusion, a laboratory parameter with a clinically significant abnormal result should be recorded in the subject's Medical Record and in the CRF as Adverse Event or Serious Adverse Event, as appropriate.

Abnormal laboratory values will be followed to provide the best possible medical care.

6.5 Criteria for stopping treatment during transfusion

Any adverse event that occurs during the transfusion will be evaluated by the treating physician and, according to the severity and safety evaluations, will decide whether or not to continue with the study.

The occurrence of anaphylaxis, which is 8 per 100,000 transfused units-UT, is defined as: acute inflammatory reaction resulting from the release of histamine and histamine-like substances by mast cells that trigger a response immune from hypersensitivity that can manifest clinically with respiratory distress, bronchospasm, dizziness, hypotension, cyanosis, loss of consciousness, edema / angioedema with or without urticaria. If the research subject presents any sign or symptom compatible with anaphylaxis he/she will be treated in a timely manner according to the Emergency protocols of Hospital Italiano. The plasma infusion will be suspended and the risk / benefit ratio of trying to transfuse it again with greater safety measures and eventually premedication will be evaluated. The subject will be monitored and followed by the Principal Investigator until the event is resolved.

Other events of very low incidence that may motivate the suspension of the transfusion: i) acute worsening of the hemodynamic and / or respiratory status caused by circulatory overload associated with transfusion (TACO); ii) respiratory and / or hemodynamic deterioration caused by transfusion-associated acute lung injury (TRALI).

TRALI is defined as an entity characterized by the development, within 6 hours of transfusion, of non-cardiogenic pulmonary edema due to endothelial damage caused by the interaction of antibodies against human lymphocytes (HLA) or against human neutrophils (HNA), or of inflammatory mediators with the pulmonary endothelium: risk factors for TRALI are: liver surgery, chronic alcohol abuse, high peak pressure in the airway during mechanical ventilation, smoking, positive fluid balance and elevated levels of Il-8 (0 , 4/100000 TU). To reduce the risk of this entity, multiparous women are excluded as donors.
Finally, transfusion-associated circulatory overload (TACO) affects, according to some studies, between 1 and 8% of transfused patients or approximately 1 in 9,177 TU. The picture consists of the onset or worsening of respiratory distress within 4-6 hours of the transfusion, elevated brain natriuretic peptide (BNP or NT-pro-BNP), increased central venous pressure, left heart failure, positive balance fluid or pulmonary edema.

6.6 Planned rescue treatment and follow-up in cases of failure or adverse events.
During the administration of plasma, the patient will be admitted to the institutional Intensive Care Unit if necessary. You will be monitored during your hospitalization by a doctor and a nurse designated exclusively for this task. In an emergency, we will proceed according to the institution's validated emergency management protocols. The patient will be cared for, giving him life support and adequate treatment. If necessary, he will be transferred to the Intensive Care Unit.

7. Ethical and regulatory considerations

All participating centers will present the project for evaluation and eventual approval by the corresponding institutional Ethics Committees. In accordance with the regulations established by the Strategic Plan to regulate the use of plasma from patients recovered from Covid-19 for therapeutic purposes defined by the Secretariat of Quality in Health of the Nation on April 16, this project will be informed to the National Directorate of Hemoderivatives of the Ministry of Health of the Nation.

Plasma donors must give their informed consent for this purpose.

The patients to be treated will also require the corresponding Informed Consent process completed with the signing of the document as a step prior to the incorporation of all patients who express their willingness to participate in the clinical trial.

Once completed, the Database of patients enrolled in the clinical trial will be registered with the National Directorate for the Protection of Personal Data, as well as the considerations about maintaining the confidentiality of the data incorporated into the text of the Informed Consent forms. They are framed within the directives established by Law 25.326 on the Protection of Personal Data. The present project as a whole will be carried out within the framework established by the Declaration of Helsinki, resolution 783/2020 (Strategic Plan to Regulate the Use of Plasma by Patients Recovered from COVID-19 for Therapeutic Purposes) and resolution 1480 / 11 of the MSN and resolution 1480/11 of the Ministry of Health of the Nation in relation to research on human beings.

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Part IC:
Study Protocol Amendments Summary

Protocol version	Ethics Committee Approval	Timing of change		Rational and key changes
		First patient enrollment (May 28th, 2020)	Interim analysis (September 1st, 2020)	
Original approval Version 2.0 Date: April 29th 2020	May 4th 2020	Before	Before	
First Amendment Version 3.0 Date: July 8th 2020	July 16th 2020	After	Before	● Added to the title: Estudio Plasmar ● Added as secondary objectives time to events: Time to the start of mechanical ventilation Time to death (days) Time to improvement of 2 points on ordinal or high scale within 30 days ● Was eliminated the analysis percentage of patients with negative PCR analyzed on day 14 ● Inclusion criteria clarified and changed: The conventional SOFA was added to the inclusion criteria and the difference of at least +2 points from the baseline for patients with an initial score other than 0 It was clarified that the O2 saturation should be at rest ● The following Exclusion Criteria have been

				added: Patients whose treatment is not going to progress at the discretion of the treating physician. Patients who after having been offered the trial more than 24 hours have passed. ● Follow-up of patients: The follow-up of the patients was clarified once they were discharged following the health recommendations. Telephone follow-up will be carried out during the corresponding visits (7, 14 and 30). At the 8-13 and 15-29 visit intervals, a minimum of two telephone calls will be made to follow up the patients. If the patient cannot attend to take samples when appropriate or it is not possible to take them at home, documented in the medical record. ● **Intervention:** The volume to be transfused to the patients was clarified according to the weight of each patient: the volume to be transfused will depend on the weight of the patient. In patients weighing less than or equal to 70 kg, the total volume to be transfused will be close to 400 ml; and in patients weighing more than 70 kg the volume will be close to 600 ml at a suggested infusion rate of 5 to 10 ml kg/h ● Was clarified The procedure of randomization by redcap and non-blind personnel ● The analysis plan was adapted to the new objectives ● Clarifications on the criteria for plasma donors in accordance with local regulations: The requirement of negative PCR for SARS-CoV-19 prior to donors was eliminated and it was clarified that it will be adapted to the official regulations in force at the

				time of donation. Pre-test for donors and selection of them according to the antibody titer (1/400) ● Clarification of plasma extraction. ● Clarification of the formation of pools ● Antibody measurement methodology: Clarified that the measurement of antibodies will be carried out by the Leloir Institute (Dr. Andrea Gamarnik Laboratory). It is clarified that prior to the infusion, an aliquot of the necessary sample to make the group and factor will also be kept for the measurement of total antibodies and neutralizers of the patients. ● Clarification of the blind opening

Part II A:

Study Statistical

Analysis Plan (version 1.0)

Primary outcome

To evaluate the difference in the ordinal outcome of 6 mutually exclusive clinical severity categories between randomization arms at day 30 after intervention [1]. The ordinal categories are: 1. Death; 2. invasive ventilatory support; 3. Hospitalized with supplemental oxygen requirements; 4. Hospitalized without supplemental oxygen requirement; 5. Discharge without full restitution; and 6. Discharged with full physical restitution.

Secondary outcomes

1. 6 categories ordinal outcome on day 7th after intervention.
2. 6 categories ordinal outcome on day 14th after intervention.
3. Time from intervention to discharge from hospital (in days).
4. Time from intervention to discharge from the ICU (in days).
5. Time from the intervention to complete restitution of physical functions (according to baseline status).
6. Time from the intervention to the start of invasive ventilatory support.
7. Time from intervention to death (in days).
8. Percentage of participants with adverse events / serious adverse events.

9. Serum D-dimer (ng/ml) plasma concentration at day 14.
10. Serum Ferritin (ng/ml) plasma concentration at day 14.
11. Plasma concentration of total antibodies on day 2 after the intervention.
12. Plasma concentration of total antibodies on day 7 after the intervention.
13. Percentage of post-transfusion specific adverse reactions between groups.

Statistical Analysis Plan

We will include all randomized patients in the primary efficacy and safety analysis (intention-to-treat analysis). We anticipate that all patients will receive the once time intervention, but if this does not occur, the possibility of performing a per-protocol analysis is contemplated.

The categorical variables will be presented as absolute and relative frequency in percentage. Continuous variables will be presented as mean and standard deviation or median and interquartile range according to the observed distribution.

In the final analysis we will consider as statistically significant all p values < 0,05 for all comparisons with the exception of the adjusted p values for the primary outcome analysis defined in the interim analysis section. The statistical analysis will be performed with the statistical software STATA version 15.1 MP - Parallel Edition (Copyright 1985-2017 StataCorp LLC - StataCorp. 4905 Lakeway Drive, College Station, Texas 77845 USA).

Primary outcome and secondary outcomes #1 and #2. Efficacy analysis

The primary objective is to evaluate the difference in the ordinal outcome with 6 mutually exclusive severity clinical categories between randomization arms at day 30 after intervention [1]. The ordinal categories are: 1. Death; 2. Invasive ventilatory support; 3. Hospitalized with supplemental oxygen requirements; 4. Hospitalized without supplemental oxygen requirement; 5. Discharge without full restitution; and 6. Discharged with full physical restitution. Considering the primary objective, we will use an ordinal regression model for proportional odds [2,3]. This model estimates a common odds ratio for the difference between the ordinal categories of the main outcome variable. The proportional odds ratio assumption will be evaluated with the Brant test (parallel regression assumption test) [4,5]. Considering potential issues referred to the proportional OR assumption, we will also test the difference in the ordinal scale at day 30 between arms using the Wilcoxon rank sum test [6].

Secondary outcomes #3 to #6. Time to event with competing events. Efficacy analysis

For time to event secondary outcomes with death as competing event (#3 to #6 secondary outcomes: discharge from hospital, discharge from the ICU, complete restitution of physical functions, start of invasive ventilatory support respectively), we will use methods to analyze right censored data. The cumulative incidence of each event will be estimated with the Kaplan Meier method for each arm of exposure. We will estimate median time to event for each exposure arm. The association between the randomized arm and each event will be evaluated using Fine and Gray regression models considering death as a competing event [7]. subHazard Ratios (sHR) will be presented with their 95% confidence intervals (95% CI) and the p values of the Wald test for each coefficient.

Secondary outcomes #7. Time to death. Efficacy analysis

For time to death, we will use conventional methods to analyze right censored data. The cumulative incidence of each event will be estimated with the Kaplan Meier method for each arm of exposure. We will estimate median time to death for each exposure arm. The association between the randomized arm and each event will be evaluated using Cox-Mantel test and Cox proportional hazards regression models. Hazard Ratios (HR) will be presented with their 95% confidence intervals (95% CI) and the p values of the Wald test for each coefficient.

Secondary outcome #8 and #13. Safety analysis

For secondary outcome #9 (adverse events / serious adverse events) and #14 (post-transfusion specific adverse reactions), logistic regression analysis will be used to evaluate the association between the randomization arm and the safety outcomes: percentage of adverse events and severe adverse events. We will provide the estimated ORs with their 95% CIs and the p values corresponding to the Wald test for each coefficient.

Secondary outcome #9 to #12

T test or Mann Whitney test will be used between randomization arms corresponding to objectives #10 to #13 according to assumptions. We will use logarithmic transformation of serum D-dimer plasma concentration, serum Ferritin plasma concentration and plasma concentration of total antibodies and linear regression models to evaluate the association between randomized arm and each concentration.

Sensitivity analysis

As a sensitivity analysis for the primary and secondary outcomes #1 and #2, the results will be presented considering the ordinal outcome as a dichotomous variable of discharge at home (with and without complete restoration of physical functions) as the outcome variable using logistic regression. The same analysis will be used to assess the secondary outcomes #1 and #2 at day 7 and 14.

Planned Subgroup efficacy analysis

The results of the subgroup analysis will be presented with the p value of the interaction test and the OR or HR calculated for each subgroup with 95% CIs. In all cases we will use regression models. We planned to perform subgroup analysis according to the following categories.

1. Participating sites
2. Age (age categories <65; >=65 & <80; >=80 / age continuous)
3. Gender
4. Time delay between initiation of symptoms and intervention (continuous / median 8 / <72 hour from initiation of symptoms)
5. Presence of comorbidities (immunosuppression, obesity, diabetes, arterial hypertension, cardiovascular disease)
6. Category NEW (<80, <72 hour from onset of symptoms, without comorbidities)
7. Baseline serum antibodies title of each participant (continuous / median)
8. Patients that received glucocorticoids

The following subgroup analysis will be performed only in the active treatment subgroup:

9. Neutralizing antibodies received (continuous / median)

Interim efficacy and safety analysis

An interim efficacy, safety, and futility analysis will be performed after 50% (166) of patients had been included in the study. The analysis will be carried out by the statistical team in a non-blind manner. The rest of the research team will remain blinded to the study arms distribution. In this analysis, the research team will eventually be asked to decide on early termination for efficacy or safety in accordance with the interim analysis results showing clear and substantial evidence. For this analysis, p values less than 0.003 are considered statistically significant for the efficacy analysis according to the strategy proposed by O'Brien and Fleming [10]. According to this strategy, in the final efficacy analysis, p values less than 0.049 will be considered statistically significant.

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Part II B:

Study Statistical

Analysis Plan (version 2.0)

Primary outcome

To evaluate the difference in the ordinal outcome of 6 mutually exclusive clinical severity categories between randomization arms at day 30 after intervention [1]. The ordinal categories are: 1. Death; 2. invasive ventilatory support; 3. Hospitalized with supplemental oxygen requirements; 4. Hospitalized without supplemental oxygen requirement; 5. Discharge without full restitution; and 6. Discharged with full physical restitution.

Secondary outcomes

1. 6 categories ordinal outcome on day 7th after intervention.
2. 6 categories ordinal outcome on day 14th after intervention.
3. Time from intervention to discharge from hospital (in days).
4. Time from intervention to discharge from the ICU (in days).
5. Time from the intervention to complete restitution of physical functions (according to baseline status).
6. Time from the intervention to the start of invasive ventilatory support.
7. Time from intervention to death (in days).
8. Time to improvement of 2 categories in the ordinal outcome or hospital discharge within 30 days.

9. Percentage of participants with adverse events / serious adverse events.
10. Serum D-dimer (ng/ml) plasma concentration at day 14.
11. Serum Ferritin (ng/ml) plasma concentration at day 14.
12. Plasma concentration of total antibodies on day 2 after the intervention.
13. Plasma concentration of total antibodies on day 7 after the intervention.
14. Percentage of post-transfusion specific adverse reactions between groups.

Statistical Analysis Plan

We will include all randomized patients in the primary efficacy and safety analysis (intention-to-treat analysis). We anticipate that all patients will receive the once time intervention, but if this does not occur, the possibility of performing a per-protocol analysis is contemplated.

The categorical variables will be presented as absolute and relative frequency in percentage. Continuous variables will be presented as mean and standard deviation or median and interquartile range according to the observed distribution.

In the final analysis we will consider as statistically significant all p values < 0,05 for all comparisons with the exception of the adjusted p values for the primary outcome analysis defined in the interim analysis section. The statistical analysis will be performed with the statistical software STATA version 15.1 MP - Parallel Edition (Copyright 1985-2017 StataCorp LLC - StataCorp. 4905 Lakeway Drive, College Station, Texas 77845 USA).

Primary outcome and secondary outcomes #1 and #2. Efficacy analysis

The primary objective is to evaluate the difference in the ordinal outcome with 6 mutually exclusive severity clinical categories between randomization arms at day 30 after intervention [1]. The ordinal categories are: 1. Death; 2. Invasive ventilatory support; 3. Hospitalized with supplemental oxygen requirements; 4. Hospitalized without supplemental oxygen requirement; 5. Discharge without full restitution; and 6. Discharged with full physical restitution. Considering the primary objective, we will use an ordinal regression model for proportional odds [2,3]. This model estimates a common odds ratio for the difference between the ordinal categories of the main outcome variable. The proportional odds ratio assumption will be evaluated with the Brant test (parallel regression assumption test) [4,5]. Considering potential issues referred to the proportional OR assumption, we will also test the difference in the ordinal scale at day 30 between arms using the Wilcoxon rank sum test [6].

Secondary outcomes #3 to #6. Time to event with competing events. Efficacy analysis

For time to event secondary outcomes with death as competing event (#3 to #6 secondary outcomes: discharge from hospital, discharge from the ICU, complete restitution of physical functions, start of invasive ventilatory support respectively), we will use methods to analyze right censored data. The cumulative incidence of each event will be estimated with the Kaplan Meier method for each arm of exposure. We will estimate median time to event for each exposure arm. The association between the randomized arm and each event will be evaluated using Fine and Gray regression models considering death as a competing event [7]. subHazard Ratios (sHR) will be presented with their 95% confidence intervals (95% CI) and the p values of the Wald test for each coefficient.

Secondary outcomes #7. Time to death. Efficacy analysis

For time to death, we will use conventional methods to analyze right censored data. The cumulative incidence of each event will be estimated with the Kaplan Meier method for each arm of exposure. We will estimate median time to death for each exposure arm. The association between the randomized arm and each event will be evaluated using Cox-Mantel test and Cox proportional hazards regression models. Hazard Ratios (HR) will be presented with their 95% confidence intervals (95% CI) and the p values of the Wald test for each coefficient.

Secondary outcomes #8. Time to combine events with 30 days right censor of death as competing events. Efficacy analysis

For time to combine events including improvement of 2 categories in the ordinal outcome or hospital discharge within 30 days, we considered deaths within 30 days, as censored at day 30 as a different approach to consider death as a competing event. Considering this, we used conventional methods to analyze right censored data [8,9]. The cumulative incidence of each event will be estimated with the Kaplan Meier method for each arm of exposure. We will estimate median time to event for each exposure arm. The association between the randomized arm and each event will be evaluated using Cox-Mantel test and Cox proportional hazards regression models. Hazard Ratios (HR) will be presented with their 95% confidence intervals (95% CI) and the p values of the Wald test for each coefficient.

Secondary outcome #9 and #14. Safety analysis

For secondary outcome #9 (adverse events / serious adverse events) and #14 (post-transfusion specific adverse reactions), logistic regression analysis will be used to evaluate the association between the randomization arm and the safety outcomes: percentage of adverse events and severe adverse events. We will provide the estimated ORs with their 95% CIs and the p values corresponding to the Wald test for each coefficient.

Secondary outcome #10 to #13

T test or Mann Whitney test will be used between randomization arms corresponding to objectives #10 to #13 according to assumptions. We will use logarithmic transformation of serum D-dimer plasma concentration, serum Ferritin plasma concentration and plasma concentration of total antibodies and linear regression models to evaluate the association between randomized arm and each concentration.

Sensitivity analysis

As a sensitivity analysis for the primary and secondary outcomes #1 and #2, the results will be presented considering the ordinal outcome as a dichotomous variable of discharge at home (with and without complete restoration of physical functions) as the outcome variable using logistic regression. The same analysis will be used to assess the secondary outcomes #1 and #2 at day 7 and 14.

Planned Subgroup efficacy analysis

The results of the subgroup analysis will be presented with the p value of the interaction test and the OR or HR calculated for each subgroup with 95% CIs. In all cases we will use regression models. We planned to perform subgroup analysis according to the following categories.

15. Participating sites
16. Age (age categories <65; >=65 & <80; >=80 / age continuous)
17. Gender
18. Time delay between initiation of symptoms and intervention (continuous / median 8 / <72 hour from initiation of symptoms)
19. Presence of comorbidities (immunosuppression, obesity, diabetes, arterial hypertension, cardiovascular disease)
20. Category NEW (<80, <72 hour from onset of symptoms, without comorbidities)
21. Baseline serum antibodies title of each participant (continuous / median)
22. Patients that received corticosteroids

The following subgroup analysis will be performed only in the active treatment subgroup:

23. Neutralizing antibodies received (continuous / median)

Interim efficacy and safety analysis

An interim efficacy, safety, and futility analysis will be performed after 50% (166) of patients had been included in the study. The analysis will be carried out by the statistical team in a non-blind manner. The rest of the research team will remain blinded to the study arms distribution. In this analysis, the research team will eventually be asked to decide on early termination for efficacy or safety in accordance with the interim analysis results showing clear

and substantial evidence. For this analysis, p values less than 0.003 are considered statistically significant for the efficacy analysis according to the strategy proposed by O'Brien and Fleming [10]. According to this strategy, in the final efficacy analysis, p values less than 0.049 will be considered statistically significant.

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Part II C:
Study Statistical
Analysis Plan
Summary of changes

We added to the original Statistical Analysis Plan (version 1.0) the statistical methods to evaluate the secondary outcome in the final version of the study statistical plan (version 2.0):

#8 Time to improvement of 2 categories in the ordinal outcome or hospital discharge within 30 days.

This secondary outcome was an addition in the final amendment of the study protocol (version 3.0).